A Study on DNA Breaks and DNA-protein Crosslinks Induced by Formaldehyde in two Types of Mice Cells

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Abstract

Objective: Previous studies have confirmed that formaldehyde (FA) is genotoxic and mutagenic to mammalian cells. FA can induce a broad spectrum of genetic effects. This study is undertaken to investigate the direct effect of formaldehyde on two types of mice cells. Methods: The single cell gel electrophoresis (SCGE) and KCl-SDS assay were used to detect the effect of FA in mice lung cells and mast cell line (P815) in vitro. Results: The effects of formaldehyde in the two types of cells were similar. The results showed that liquid formaldehyde induced DNA breakage at 5 μM, 25 μM ($P<0.01$, compared with control group) and the DNA-protein crosslinks were found at high concentrations (125 μM, $P<0.05$; 625 μM, $P<0.01$) Conclusions: The results showed that formaldehyde caused DNA breaks and crosslinks for different types of mice cells.

Keywords

formaldehyde, genotoxicity, DNA-protein crosslinks, DNA single strand breaks

Introduction

Formaldehyde (FA) is a genotoxic and mutagenic substance. In 2004, a WHO working group, IARC (International Agency for Research on Cancer) concluded that FA is carcinogenic to humans (group 1A) after reevaluating the available evidences on the carcinogenicity of FA. Generally speaking, formaldehyde is a colorless, highly flammable gas. Nowadays, more attention has been paid on the indoor air pollution and formaldehyde is regarded as an important indoor air chemical pollutant for its extensive sources, high level, long duration and high toxicity. Epidemiological studies have shown that formaldehyde may be responsible for several diseases on human. At relatively low level (0.5 mg/m^3), formaldehyde can induce eyes and upper respiratory irritancy [1]; occupational exposure to high level of gaseous formaldehyde increases the risks of asthma [2]. It has also been reported that formaldehyde affects immune system and pulmonary function [3-4]; many studies *in vitro* have shown that FA has extensive genotoxicity, including DNA-protein crosslinks (DPC) and DNA single strand breaks (DSSB) [5]. In this study, KCl-SDS assay and single cell gel electrophoresis (SCGE) were used to detect the direct effect of FA on mice lung cells and mast cell line (P815) *in vitro*.

Materials and methods

Reagents and apparatus

10% formalin, calf thymus DNA and fluorescence dye Hoechst 33258 were purchased from Sigma. RPMI1640 and fetal bovine serum (FBS) were purchased from Gibco. SDS and proteinase K were purchased from Merk. Acridine Orange, Triton X-100 and N-lauroylsarcosine were purchased from Amresco. Normal melting agarose and low melting agarose were purchased from Promega. Cell counting 8 kits was purchased from Dojindo.

Apparatus include CO_2 incubator (Thermo Forma), Centrifuge (Eppendof-5415R); Nikon fluorescence microscope (E600); Fluorescence spectrophotometer (RF-5301PC, Shimadzu)

Cell separate and culture

The mice lung cells were separate from Kun Ming male mice supplied by the Experimental Animal Center of Hubei. The P815 cell line was purchased from the Chinese center for cell culture collection located in Wuhan University, China. The mast cells were cultivated in RPMI 1640 supplemented with 10% FBS in a humidified atmosphere of 95% air, 5% CO_2 at 37℃. The medium was changed every other day.

Exposure of cells to FA

The separated or cultured cells were beaten gently to suspend cells with RPMI 1640 to give a concentration of about 1×10^6 cells/ml. Then the suspended cells were distributed to Eppendof tubes according to 0.5 ml per tube. FA was added into each tube to make the final concentration consistent with the design in advance. After that, the tubes with cells were incubated for 1 h at 37℃.

Single cell gel electrophoresis (Comet assay)

Single cell gel electrophoresis is also named comet assay. It was initiated developed by Ostling and Johanson in 1984,

and was modified by Singh et al to improve the sensitivity of detection of DNA damage in 1988. In this study, comet assay was performed to detect FA-induced DNA damages according to the protocol [6] modified by Tice R.R. After lysis (2.5mM NaCl, 100mM EDTA, 10mM Tris-HCl [pH=10], 1% Triton X-100, and 10% DMSO) at 4 ℃ for 2 h, the slides were treated with 140μl of proteinase K (10 mg/ml) at 37 ℃ for 2 h. The slides were then placed in alkaline solution (300 mM NaOH, 1 mM EDTA, pH=13) for 20 min, and electrophoresis was conducted for 20 min at 17 V, 240mA. All technical steps were conducted protected from light. After electrophoresis, the slides were neutralized with 0.4 M tris-HCl, pH 7.5 for three times for 5min, and were then stained with A. O (20 μg/L) for 3 min. 50 cells in two slides were analyzed by the use of fluorescent microscope (WH-2, Olympus) and image analysis software (CASP, from www.casp.of.pl). Tail moment and tail DNA% were taken as the parameters for DNA damage [6].

KCl-SDS assay

The KCl-SDS assay was initially developed by Liu et al and modified by Zhitkovich and Costa in 1992 for detecting DPC in whole cells. The method utilizes harsh treatments to dissociate non-covalent DNA-protein binding (2% SDS, heat at 65 ℃) and selectively precipitates stable DNA-protein complexes by adding KCl [7]. In this study the KCl-SDS assay was applied to detect FA-induced DPC according to Zhitkovich and Chakrabarti with some modifications [8].

Statistical analysis

All of the data was analyzed by software Origin 5.0. Student's t-test was applied to evaluate the significance of the differences in the results between treated and control groups. A level of $P<0.05$ was considered to be statistically significant.

Results and analysis

The image of the experiment results

Figure 1-a and 1-b showed the results of comet assay of the cells of mice and the image after analyzed by CASP image analysis software.

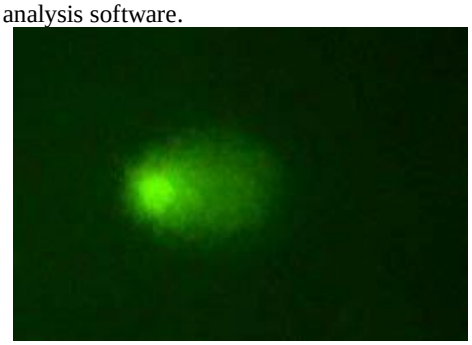


Fig 1-a. mice cell exposed to FA showed a comet character

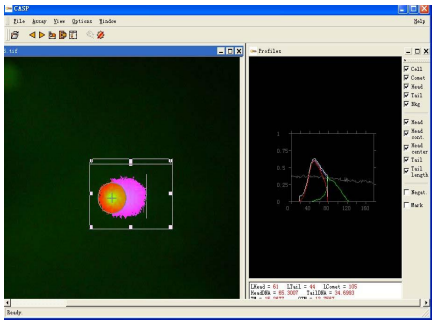
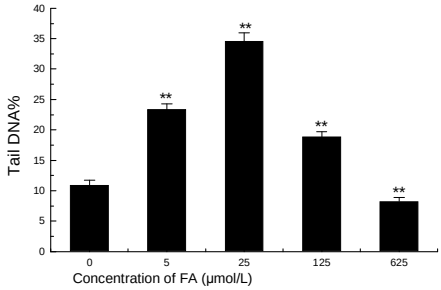


Fig 1-b. CASP image analysis software analysis result for fig 1.

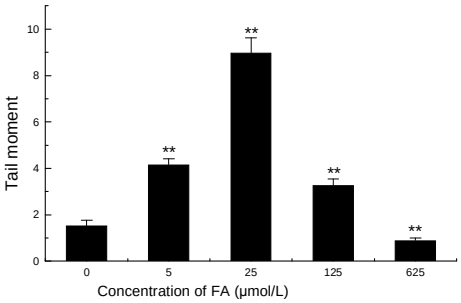
FA-induced DSSB and DPC in lung cells of mice

The effect of FA-induced DSSB and DPC in mice lung cells was examined. Figure 2-a and 2-b showed the results of DSSB. The results showed that FA could induce DSSB significantly at 5μM and 25μM in mice lung cells ($P<0.01$, compared with control group). As the increase of FA concentrations, the effect of DSSB is decreased rapidly (125 and 625μM compared with 25μM, $P<0.01$).

Figure 3 showed the result of DPC, and there was no significant difference in the DPC coefficient between the groups treated with 5μM, 25μM of FA and control group. But there was significant difference between the group treated with 125, 625μM of FA and control group ($P<0.01$).



2-a



2-b

*Figure 2: The effect of FA-induced DNA damage in mice lung cells. 2-a for tail DNA% and 2-b for tail movement (**: P<0.01, group with 5 and 25μM FA compared with 0 control; Groups with 125 and 625μM FA compared with group with 25μM FA)*

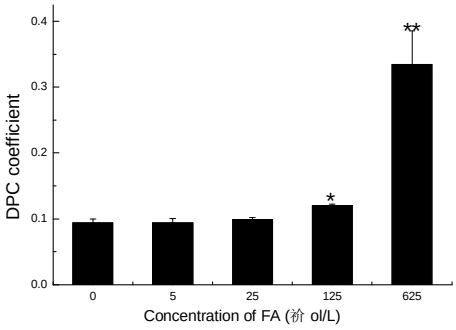
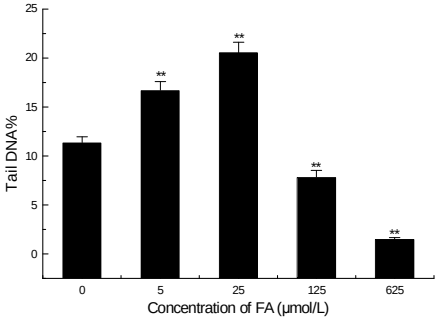


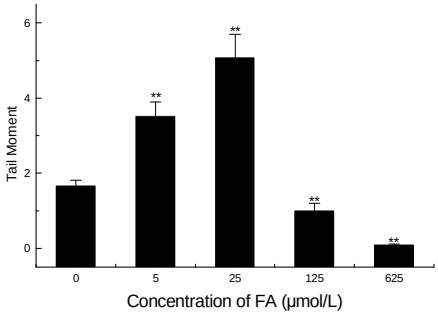
Figure 3: The effect of FA-induced DPC in mice lung cells. (: P<0.05; **: P<0.01, FA treated groups compared with control group)*

FA-induced DSSB and DPC in P815 cell

The effect of DSSB and DPC on P815 cells after FA exposure is illustrated in figure 4 and 5. Similar trend was observed in P815 cell line as in the isolated mice lung cells.



4-a



4-b

*Figure 4: The effect of FA-induced DNA damage in P815 cell line. 3-a for tail DNA% and 3-b for tail movement (**: P<0.01, group 5 and 25 μM FA compared with 0 control; Groups 125 and 625μM FA compared with group with 25μM FA)*

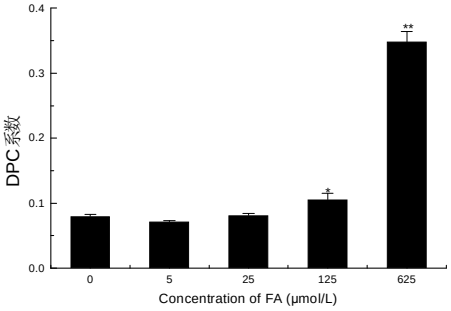


Figure 5: The effect of FA-induced DPC in P815 cell line. (: P<0.05; **: P<0.01, FA treated groups compared with control group)*

Discussions

Formaldehyde is a very common indoor air pollutant with many sources and found in almost all homes and buildings. Inhaled formaldehyde can cause both short-term irritant effects and long-term health effects, such as asthma [9]. Studies also indicated that tumors induced by FA are usually observed in the organs and tissues exposed to gaseous FA [10]. As a key event of tumors induced by FA, DPC is considered to be the primary and directly genotoxic effect of FA [11-12]. DNA breaks, including DNA single strand breaks and DNA double strands breaks, are usually regarded as a biomarker of the phenomenon that cell life-force is threatened and the integrality of gene is destroyed [13]. There may be 4 molecular mechanisms to explain the genetic toxicity of FA: firstly is the breakage of chromosome and sister chromosomes changes in cell division [2]; secondly, FA acts as oxidant which could oxidize DNA molecule directly [13], to form the 8-oxoguanine [14], to induce DNA base mutation or make DNA breakage and DNA-DNA, DNA-Protein molecule crosslink; The third one is inactivation of antioxidant enzymes by FA, which may result in the oxidation of DNA molecule indirectly[15]. For instance, FA could inactivate superoxide dismutase (SOD), which may inhibit the clean of free oxygen radicals in cells and the organs, and may induce DNA molecules oxidation indirectly. Furthermore, as indicated by a research [11], the genetic toxicity of FA may have close relationship with p53 gene mutation and mutative p53 protein. It has been pointed out that p53 is the key protein for cancer control and its function is lost by mutation.

In this study, the results of SCGE showed that liquid FA could induce DSSB in two cell lines at low concentrations.

FA resulted in a significantly increased tail moment and tail DNA% at 5μM, 25μM ($P<0.01$). However, FA caused a concentration dependent decrease of tail moment and tail DNA% in DNA migration at 125μM, 625μM, which suggested that FA may induce DNA crosslinks at this concentration. From the result of KCl-SDS in two cell lines it can be seen that there was no significant difference in the DPC coefficient between the groups treated with 5μM, 25μM of FA and control group. But there was significant difference between the group treated with 125μM, 625μM of FA and control group (125μM， $P<0.05$； 625μM， $P<0.01$), which demonstrated that FA induced DPC in two cell lines at high concentrations.

Conclusion

In conclusion, FA at different concentrations can induce different DNA damages, leading to DNA strands breaks at low concentrations and DNA crosslinks at high concentrations, and the effect of formaldehyde inducing DPC and DSSB in mice lung cells and mast cell were the similar. It can also be concluded that KCl-SDS assay and single cell gel electrophoresis (SCGE) were useful methods to study the genotoxicity of FA on DNA.

Acknowledgements

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